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Comparison of cadmium pathways in a high Cd accumulating cultivar versus a low Cd accumulating cultivar of *Theobroma cacao* L.

Hester Blommaert^{a,b,*}, Anne-Marie Aucour^c, Matthias Wiggenhauser^d, Sylvain Campillo^a, Claudia Moens^e, Hiram Castillo-Michel^f, Jesse Dekeyrel^e, Pathmanathan Umaharan^g, Erik Smolders^e, Géraldine Sarret^{a,**}

^a Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, IRD, Université G. Eiffel, ISTERre, Grenoble, France

^b Institut Néel, CNRS, Université Grenoble Alpes, 25 Avenue des Martyrs, 38042, Grenoble, France

^c Université de Lyon, Université Lyon 1, ENS de Lyon, CNRS, UMR 5276 LGL-TPE, F-6922, Villeurbanne, France

^d Institute of Agricultural Sciences, ETH Zurich, Eschikon 33, CH-8315, Lindau, Switzerland

^e Division of Soil and Water Management, Department of Earth and Environmental Sciences, KU Leuven, Belgium

^f ESRF, The European Synchrotron, CD 40220, Grenoble Cedex 9, 38043, France

^g Cocoa Research Centre, University of the West Indies, St. Augustine, Trinidad and Tobago

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ABSTRACT

Understanding cadmium (Cd) pathways in cacao trees is critical for developing Cd mitigation strategies. This study investigates whether Cd uptake and translocation mechanisms differ between a low and a high Cd-accumulating cacao cultivar. We sampled three replicate trees of each cultivar, and a grafted cultivar that shared the same scion as the low Cd accumulator but had a different rootstock. All cultivars grew in the same field with similar bioavailable soil Cd. We utilized Cd stable isotope analyses to trace Cd pathways within the trees, complemented by micro-scale imaging of Cd distribution in leaves and branches, and nutrient and Cd quantification across tree organs. The high Cd accumulator exhibited 2.9-fold higher Cd uptake than the low Cd accumulator, while the grafted cultivar showed 1.7-fold higher uptake. These differences matched Mn uptake. The $\delta^{114/110}\text{Cd}$ values of organs increased in the order: roots \leq nibs, young leaves \leq branches \leq pod husks $<$ mature leaves for the high and grafted cultivar, and nibs \leq roots \leq branches \leq pod husks $<$ mature leaves for the low cultivar. The enrichment in heavy isotopes correlated with a progressive retention of Cd compared to Mn along the xylem pathway from roots to branches to leaves. The differences in Cd isotope compositions between cultivars indicate that there are differences in translocation processes, yet they did not affect the relative Cd internal distribution. Cd mass balances and internal translocation factors (ITF) corroborated that differences in nib Cd concentrations among cultivars were primarily due to uptake rather than translocation.

1. Introduction

Cadmium (Cd) is a toxic trace metal associated with significant health risks to humans (FAO/WHO, 2010; WHO, 2019). Many countries have established limits on permissible Cd levels in food products to safeguard public health (Zhao et al., 2022). In 2014, a Joint FAO/WHO Expert Committee on Food Additives (JECFA) reviewed Cd concentrations in various food items. This panel estimated that chocolate products are a significant source of Cd in the European diet, especially for children (EFSA, 2012). Consequently, the European Commission set limits

for Cd in chocolate and cacao powder, ranging from 0.10 to 0.80 mg Cd kg⁻¹ (European Commission, 2014). Other regulatory bodies worldwide have adopted similar standards (Australia New Zealand Food Standards Code, 2017; Codex Alimentarius Commission, 2018; Mercosur, 2011; Ministry of Health of the Russian Federation, 2011). These regulations have resulted in restricting market access for cacao and chocolate products that do not comply with these regulations. The regulations are currently threatening the economic sustainability of cacao production, especially in South-American countries (Meter et al., 2019; Vander-schueren et al., 2021; Vázquez-deCastro et al., 2024).

* Corresponding author: Université Grenoble Alpes, Université Savoie Mont Blanc, CNRS, IRD, Université G. Eiffel, ISTERre, Grenoble, France.

** Corresponding author.

E-mail addresses: hester.blommaert@univ-grenoble-alpes.fr (H. Blommaert), geraldine.sarret@univ-grenoble-alpes.fr (G. Sarret).

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Several strategies have been proposed to reduce Cd concentrations in chocolate products (Meter et al., 2019; Vanderschueren et al., 2021). Among them are agronomic approaches such as the application of soil amendments (Argüello et al., 2023; Ramtahal et al., 2019). Alternatively, genetic approaches have been advocated based on observation of differences in Cd accumulation between cacao cultivars in the field (Arévalo-Gardini et al., 2017; Engbersen et al., 2019; Lewis et al., 2018). These studies have prompted interest in the selection of low Cd accumulating cultivars, and/or the use of low Cd accumulating rootstocks in grafting. In the latter, cacao shoots can be grafted on a rootstock with low Cd uptake and/or translocation to the fruit (Fernández-Paz et al., 2021). For the development of both approaches, understanding the Cd accumulation pathways in more detail, particularly the differences between high and low Cd accumulating cultivars, as has been done for other crops (for example in wheat (Harris and Taylor, 2004), hot pepper (Xin et al., 2014), radish (Xin et al., 2017), and sweet potato (Huang et al., 2019)), is critical.

In a previous study, we investigated Cd pathways and storage forms in a high Cd accumulating cultivar (NA 312) (Blommaert et al., 2022, 2024a). In short, Cd was weakly retained in roots, and the majority was translocated to the shoot. Among the various organs studied (Fig. 1), branches exhibited the highest Cd concentrations. Imaging combined with stable isotope analyses (i.e. isotope fractionation) showed a xylem to phloem transfer of Cd within the rays of branches and part of Cd was bound to Ca-oxalate crystals. Further along the transpiration pathway to the mature leaves, Cd was progressively retained in the bark of the branches. The isotopic signature of the testa (seed coat) and pod husk suggested that these organs received Cd from both xylem and phloem. Interestingly, the heavier isotopic signature of the nibs (seeds) suggested that Cd was loaded mostly from the phloem. An additional isotope tracer experiment in cacao seedlings confirmed that Cd was indeed mobile in the phloem, and showed that the Cd xylem and phloem mobility were intermediate between hyperaccumulator plants and non-tolerant plants (Blommaert et al., 2024b). More specifically, in non-tolerant plants, there is a strong retention of Cd in the roots, i.e. a low xylem mobility, but a high phloem mobility allowing redistribution within the plant

(Page and Feller, 2005). On the contrary, in hyperaccumulators, Cd xylem mobility is high and Cd is rapidly transported to the shoots. Once in the leaves, it is not or slowly redistributed via the phloem within the shoots (Wei et al., 2014). Cacao exhibits a behavior intermediate between these model plant species.

Given these initial findings, there is a need to confirm Cd pathways in other cacao genotypes, and/or to identify processes that control the mobility of Cd within trees. The objectives of this study were to compare and corroborate the Cd uptake and translocation mechanisms between the high Cd accumulating cultivar, previously characterized (Blommaert et al., 2022), with a low Cd accumulating cultivar. Cd uptake refers to the total absorption of Cd from the soil into the trees, and Cd translocation refers to the movement of Cd from the roots to other parts of the tree through the plant's vascular system. Additionally, we compared the low Cd accumulating cultivar with a grafted cultivar that had the same scion as the low Cd accumulating cultivar but was propagated on another rootstock. We included this grafted cultivar as a point of comparison, to corroborate the isotope analyses in the other cultivars, and to provide first indications on the effect of rootstock on Cd uptake and translocation.

2. Materials and methods

We collected samples in January 2020 at the International Cocoa Genebank, Trinidad (ICGT). The ICGT maintains a cacao collection in the field with approximately 2400 cacao genotypes, also further described as cultivars. We selected two cultivars with contrasting Cd levels in beans and leaves (Lewis et al., 2018). NA 312 of the genetic group *Nanay*, had high Cd levels in beans and leaves and was thus considered a high Cd accumulating cultivar. We already characterized the Cd accumulation of this cultivar in a previous study (Blommaert et al., 2022). The other cultivar, further mentioned as the low Cd accumulator, was CL 10/3 from a hybrid group referred to as *Refractario*. Additionally, there was also a plot of trees of CL 10/3 that were later introduced in the gene bank and were grafted on a TSH 919 rootstock (Trinidad Selected Hybrids are a group of clonal cultivars developed in

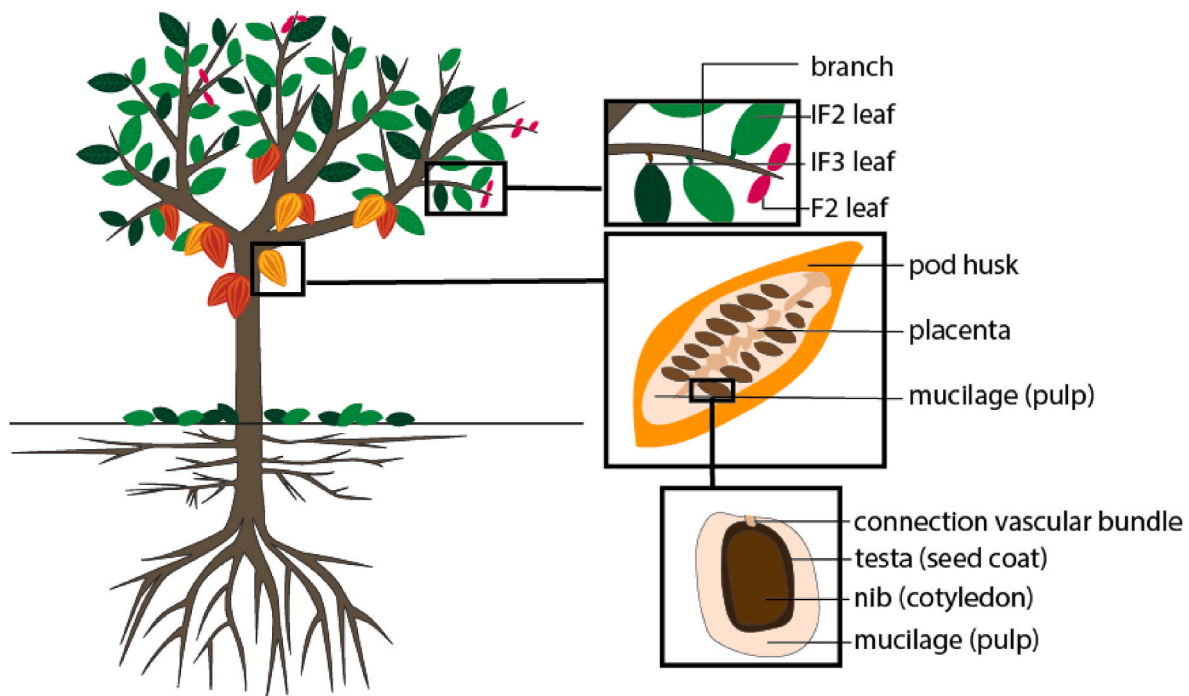


Fig. 1. Terminology of the studied organs of the cacao trees in this study. IF2 and IF3 leaves are mature leaves, whilst F2 leaves are young, developing leaves (see section 2.1). The cacao fruit or pod is made up of a large woody outer pod husk, filled with 20–50 cacao beans (seeds). The cacao bean consists of a nib and an outer shell, the testa. The nib is the only part of the cacao bean that is retained during chocolate processing.

Trinidad and Tobago). These grafted trees are neither classified as a high Cd accumulator, nor as a low Cd accumulator. In this study, we will refer to this treatment as the grafted cultivar. In brief, the experiment consisted of three cultivars with each three biological replicates: high Cd accumulator (T2, T4, T6), low Cd accumulator (T1, T3, T15), and grafted (T1, T2, T3). The numbers correspond with the respective trees on the field (there were more than three trees on each plot). The biological replicates were genetically identical trees, confirmed by DNA fingerprinting. In this study, we include data on total elemental concentrations and Cd isotope ratios already published for the high Cd accumulator (Blommaert et al., 2022) to facilitate the comparison among the three cultivars. Results that are already published are indicated in each table.

2.1. Soil and tree sampling

The procedure for soil and tree sampling at the ICGT has been extensively described for the high Cd accumulator in Blommaert et al. (2022). We used similar procedures for the low Cd accumulator and the grafted cultivar. Briefly, topsoil (0–20 cm), subsoil (20–40 cm), roots, branches, interflush-2 (IF2) leaves (mature green leaves with partly green partly brown stem (Greathouse et al., 1971)), interflush-3 (IF3) leaves (mature green leaves with brown stem) were collected for all replicates of the three cultivars (Fig. 1). In addition, leaves in the flushing 2 phase (F2) were sampled for the low Cd accumulator and grafted cultivar. F2 leaves are young, developing leaves in their expanding phase and are characterized by a red color (Greathouse et al., 1971). In the high Cd accumulator, no F2 leaves were available at the time of sampling. For all trees, healthy fruits were collected, and due to the limited number of fruits on the trees, mature as well as intermediate mature and immature pods were collected. The grafted cultivar bore only one fruit in one tree at the time of sampling. The organs sampled and the corresponding number of biological replicates are shown in Table 1.

All plant samples (roots, branches, leaves, pods) were washed and rinsed with deionized water, and then dried with paper towels. The intact pod husk was opened and all different tissues (pod husk, placenta, testa, nib (Fig. 1)) were separated and weighed. For imaging analyses, intact branches (diameter 2–5 mm) were oven-dried and stored for further sample preparation. Intact pieces of the leaf blade, cut around a vein, were put in an O.C.T. (Optimal Cutting Temperature) resin (Tissue-Tek® O.C.T. Compound (polyvinyl alcohol and carbowax). These samples were then rapidly frozen in isopentane (cooled by liquid nitrogen

(LN₂)) to preserve their fresh state, following the method described by Castillo-Michel et al. (2017). Aliquots of the samples for concentration and isotope analyses were weighed for fresh weight/dry weight determination, homogenized, oven-dried (65 °C), and shipped. The roots, branches, leaves, pod husks, and testa were ground to powder with a mechanical grinder (Pulverisette7, Fritsch), while the nibs were ground with a coffee grinder.

2.2. Chemical analyses

2.2.1. Soil and plant properties

For the plant elemental concentration analyses, 50 mg of the powder was digested in concentrated nitric acid (2 mL Suprapur HNO₃) in an open digestion block for 8 h at a maximum temperature of 130 °C. For the soils, an aliquot was taken from the samples after they were dissolved with aqua regia + HF for total elemental analyses and for Cd isotope analyses (see next section). The total elemental composition of the digest was measured by ICP-MS (Agilent 7700x, Agilent Technologies) and ICP-OES (iCAP 7400 series, Thermo Scientific). For quality control, we determined elemental concentrations in the certified reference materials BCR-142R (light sandy soil), NIST 1573a (tomato leaves), and NIST 2384 (baking chocolate). The Cd recovery (%) (equal to [measured concentration]/[reference concentration]*100) of these samples ranged from 89 to 91%. A full overview of the quality control, including other elements than Cd, is given in the SI of Blommaert et al. (2022).

In addition, we determined soil properties such as pH, organic carbon (expressed in %OC), soil effective cation exchange capacity (eCEC), ammonium oxalate extractable aluminum, iron and manganese, and 0.05 M Ca(NO₃)₂ (5 g soil: 50 mL Ca(NO₃)₂)-extractable minerals with the protocols described in Blommaert et al. (2022).

2.2.2. Cadmium isotope analyses

Prior to the isotope analysis, plant (200 ± 20 mg) and soil (400 ± 20 mg) samples were digested and subsequently purified using an anion exchange chromatography procedure as described in Blommaert et al. (2022). Cadmium isotope compositions were measured with a multiple collector ICP mass spectrometer (MC-ICP-MS, Neptune Plus, Thermo Scientific). To correct for instrumentally induced mass bias, 'standard-sample bracketing' was applied, and standard and samples were doped with silver (using Cd:Ag ratio of 2:1). Frequent measurements of a certified isotope standard and soil and plant standards for interlaboratory comparison revealed robust precision and accuracy for Cd

Table 1

Total soil Cd, 0.05 M Ca(NO₃)₂-extractable soil Cd, and plant organ Cd concentrations for the three sampled cultivars.

		Cd concentration (mg kg ⁻¹ DW)								
		High			Low			Grafted		
		Mean	SD	n	Mean	SD	n	Mean	SD	n
Topsoil	Total ^S	0.28^a	0.06	3	0.19^a	0.02	3	0.32^a	0.07	3
	Ca(NO ₃) ₂ ^S	0.06^a	0.02	3	0.07^a	0.01	3	0.10^a	0.01	3
Subsoil	Total ^S	0.10^a		1	0.10^a		1	0.10^a		1
	Ca(NO ₃) ₂ ^S	0.03^a	0.01	2	0.03^a	0.01	2	0.04^a	0.01	3
Roots		3.21^a	0.87	3	1.11^b	0.37	3	1.75^{ab}	0.37	3
F2 leaves*					1.50	0.32	2	0.50		1
IF2 leaves**		3.49^a	1.12	3	0.99^b	0.21	3	2.27^{ab}	0.18	3
IF3 leaves**		3.10^a	0.35	3	1.36^b	0.45	3	3.67^a	0.70	3
Branches		6.11^a	0.44	3	2.27^c	0.77	3	3.89^b	0.51	3
Nib		2.00^a	0.27	3	0.54^b	0.18	3	1.42		1
Testa		2.35^a	0.40	3	0.67^b	0.23	3	0.97		1
Pod husk		2.92^a	0.72	3	0.87^b	0.54	3	1.59		1
Placenta		1.24^a	0.34	3	0.25^b	0.09	3	0.40		1

The data in italic is also published in Blommaert et al. (2022). The immature, intermediate, mature samples of the fruit organs were pooled together for each biological replicate, i.e. individual tree. The means and standard deviations (SD) were calculated with the biological replicates. Letters a, b, c denote different statistical groups with the post hoc Tukey HSD test ($p < 0.05$) within the same row. Only one tree of the grafted cultivar produced mature pods at the time of sampling.

^STotal digestion (aqua regia with subsequent HF digestion).

^S 0.05M Ca(NO₃)₂-extract: (5g soil: 50 mL Ca(NO₃)₂). *Flushing 2 leaves are young, developing leaves. **Interflush-2 and interflush-3 leaves are mature green leaves.

concentration and Cd isotope measurements (see SI Blommaert et al. (2022)).

2.3. Laser-ablation ICP-MS imaging (LA-ICP-MS)

We selected two IF3 leaves for elemental mapping with LA-ICP-MS (one replicate for the low Cd accumulator (T3), and one replicate for the high Cd accumulator (T2)). In addition, we analyzed two branch cross-sections of two biological replicates (T3 and T15) of the low Cd cultivar. We prepared the thin sections of leaves by cryosectioning the frozen-hydrated leaf sections that were embedded in O.C.T. We performed the cryosectioning with a cryomicrotome (Leica CM, 1950), as detailed in Castillo-Michel et al. (2017). The sections were freeze-dried in a vacuum for 20–30 min, then moved to a cabinet for 10 min to warm gradually. Afterwards, the sections were air-dried and finally attached to a glass slide with Kapton tape prior to measurement. The branches with a diameter of ca. 5 mm were oven-dried (60 °C), cut with a microtome blade, and placed on a glass slide with Kapton tape prior to measurement. For more information on the operating conditions and parameters of LA-ICP-MS measurement, the reader is referred to Blommaert et al. (2022).

2.4. Calculations and statistics

Mass balances were estimated to determine the Cd uptake (i.e. soil to cacao-tree transfer of Cd). We estimated Cd mass balances under the assumption that the biomass of all sampled trees was equal. The biomass distribution used in this study was based on a study that weighed cacao trees that were 10 years old (Nurafiza et al., 2017). The average biomass per organ of 20 cacao trees was as follows: roots: 31.50 kg, stem: 22.50 kg, branches: 61.90 kg, leaves IF2: 7.75 kg and leaves IF3: 7.75 kg. The trees in the study of Nurafiza et al. (2017) were sampled outside of cacao peak fruiting season. Therefore, to include the fruits in this study, the dry weights of the sampled fruits (51 ± 18 g DW [average \pm SD, $n = 11$], 1 or 2 fruits on each tree), were added to calculate the Cd mass distribution in the trees. Since the stems were not sampled, the same Cd concentration as in the branches was assumed.

Isotope ratios of $^{114}\text{Cd}/^{110}\text{Cd}$ in the soil and cacao samples were expressed relative to the $^{114}\text{Cd}/^{110}\text{Cd}$ isotope ratio of isotope reference material NIST 3108 using the δ notation:

$$\delta^{114/110}\text{Cd} (\%) = \left[\frac{\left(\frac{^{114}\text{Cd}}{^{110}\text{Cd}} \right)_{\text{sample}}}{\left(\frac{^{114}\text{Cd}}{^{110}\text{Cd}} \right)_{\text{NIST3108}}} - 1 \right] \times 1000 \quad (1)$$

The weighted average Cd isotope composition and Cd concentration of in the entire tree, shoot, and fruit was calculated as a weighted arithmetic mean (shown for a tree), with the Cd mass (mg) as weighing factor:

$$\delta^{114/110}\text{Cd}_{\text{tree}} = \frac{\sum_i m_i c_i \delta^{114/110}\text{Cd}_i}{\sum_i m_i c_i} \quad (2)$$

where m_i denotes the dry weight (in g), c_i the Cd concentration (in mg kg^{-1}) and δ_i the corresponding isotope composition of the different cacao organs i (roots, stem, branch, leaves and fruits).

The difference in isotope composition Δ_{A-B} (often mentioned as apparent isotope fractionation) between two compartments A and B is expressed as:

$$\Delta^{114/110}\text{Cd}_{A-B} = \delta^{114/110}\text{Cd}_A - \delta^{114/110}\text{Cd}_B \quad (3)$$

Transfer factors (TF) indicate to what extent a plant accumulates Cd in its organs and were calculated as the ratio of the Cd concentration (Cd) in either the plant root/leaf or the edible part (mg (kg DW) $^{-1}$) to the total Cd concentration of the soil Cd_{soil} (mg (kg DW) $^{-1}$). For instance:

$$\text{TF}_{\text{nib}} = \frac{Cd_{\text{nib}}}{Cd_{\text{soil}}} \quad (4)$$

Similarly, internal translocation factors (ITF) were calculated to indicate how Cd is distributed within cacao trees. They were calculated as the ratio of the Cd concentration in an organ, over the Cd concentration in another organ (mg (kg DW) $^{-1}$), as shown in next example.

$$\text{ITF}_{\text{nib-branch}} = \frac{Cd_{\text{nib}}}{Cd_{\text{branch}}} \quad (5)$$

The standard deviation (SD) reported in this study was calculated with biological replicates, where one biological replicate is defined as an individual tree. All technical replicates or sampling replicates (e.g. different fruits) were first averaged per tree before calculating the SD. Differences in isotope composition, TF and ITF were always calculated using data from the corresponding tree (paired data). A one-way analysis of variance (ANOVA) was conducted to test whether the means of different groups/cultivars (e.g., high, low, grafted) differed significantly from each other. Posthoc Tukey HSD tests were used when ANOVA revealed significant differences in the comparison of means (level of significance: $p < 0.05$). The data sets were first tested for equality of variances with the O'Brien and Brown-Forsythe test. Additionally, the normal distribution of residuals was tested with the Shapiro-Wilk test. If these prerequisites were not fulfilled, the data were log-transformed prior to conducting an ANOVA or linear regression analysis. Such data transformation was required for the molar ratios of the nutrients versus Cd in the fully digested samples. All statistical analyses were conducted with JMP® Pro version 17.0.0 (SAS Institute, 2022).

3. Results

3.1. Soil characteristics of the plots of the cultivars

The soil from the plot of the high Cd accumulator was characterized in Blommaert et al. (2022) as strongly acidic, with relatively low % OC and total Cd compared to other cacao plantations (Vanderschueren et al., 2021). The total topsoil Cd (0.26 ± 0.07 mg Cd kg^{-1} [average \pm SD, $n = 9$]), pH (4.20 ± 0.09), soil organic carbon (2.10 ± 0.38 %), and $\text{Ca}(\text{NO}_3)_2$ -extractable Cd (0.08 ± 0.02 mg Cd kg^{-1}) did not differ significantly among the soils of the different plots ($p < 0.05$) (Table 1 and S2). The ammonium oxalate extractable Mn did not differ among the topsoils of the different plots (Table S2). However, the topsoil of the grafted cultivar had a slightly higher CEC, higher content of exchangeable K and Ca, ammonium oxalate extractable P, Fe and Al, as well as a lower $\text{Ca}(\text{NO}_3)_2$ -extractable Mn content than the high Cd accumulating cultivar.

In all plots, the subsoil was more acidic than the topsoil (3.79 ± 0.09), and lower in total Cd (0.10 ± 0.01 mg Cd kg^{-1}) and $\text{Ca}(\text{NO}_3)_2$ -extractable Cd (0.03 ± 0.01 mg Cd kg^{-1}). Most subsoil characteristics were similar for the three cultivars. However, the subsoils of the grafted cultivars again had a higher CEC, and a higher $\text{Ca}(\text{NO}_3)_2$ -extractable Zn compared to the subsoils of the high and low Cd accumulating cultivars (Table S2).

3.2. Cadmium uptake and distribution in the three cultivars

In all three cultivars, the tree organs had 2 to 20-fold higher Cd concentrations than the topsoil (Table 1). The Cd concentrations in the trees increased in the order: fruits (testa, pod husk, and nib) < roots \leq leaves < branches. In all organs, Cd concentrations were significantly higher in the high Cd accumulator than those in the low Cd accumulator, while those in the grafted plants were intermediate to the high and low Cd accumulator. The transfer factors from soil to organ revealed significant differences in Cd uptake between the high and the low cultivar (Table 2). Using Cd mass balance calculations (Fig. 2), we estimated that total Cd uptake was in average 2.7 times higher in the high Cd

Table 2
Cadmium transfer and internal translocation factors for the different cultivars.

Transfer and internal translocation factors						
	High		Low		Grafted	
	Mean	SD	Mean	SD	Mean	SD
Transfer factor						
root/topsoil [§]	<i>11.5^a</i>	1.11	<i>5.85^b</i>	1.9	<i>5.59^b</i>	1.66
IF2 leaf/topsoil [§]	<i>13.1^a</i>	5.23	<i>5.16^b</i>	0.83	<i>7.20^{ab}</i>	1.04
nib/topsoil [§]	<i>7.38^a</i>	0.80	<i>2.89^b</i>	0.83	<i>5.35</i>	.
Internal translocation factor						
branch/root	<i>1.97</i>	0.22	<i>2.04</i>	0.05	<i>2.30</i>	0.22
nib/root	<i>0.64</i>	0.12	<i>0.49</i>	0.10	<i>0.69</i>	.
IF2 leaf/root	<i>1.17</i>	0.58	<i>0.91</i>	0.16	<i>1.33</i>	0.25
nib/branch	<i>0.33</i>	0.03	<i>0.24</i>	0.04	<i>0.32</i>	.
IF2 leaf/branch	<i>0.58</i>	0.21	<i>0.45</i>	0.07	<i>0.58</i>	0.07
nib/IF2 leaf	<i>0.60^a</i>	0.20	<i>0.54</i>	0.08	<i>0.64</i>	.
IF3 leaf/IF2 leaf	<i>0.93^b</i>	0.17	<i>1.36^{ab}</i>	0.20	<i>1.63^a</i>	0.36

The data in italic is published in Blommaert et al. (2022). Letters a, b denote different statistical groups with the posthoc Tukey HSD test ($p < 0.05$) among cultivars. [§]Total digestion of the soil was conducted with aqua regia with subsequent HF digestion. Transfer factor (TF) = ratio of the Cd concentration in a plant organ (mg (kg DW)^{-1}) to the total Cd concentration of the soil (mg (kg DW)^{-1}). Internal translocation factor (ITF) = ratio of the Cd concentration in an organ (mg (kg DW)^{-1}) over the Cd concentration in another organ (mg (kg DW)^{-1}). These ratios were calculated per individual tree, and subsequently averaged per cultivar (paired data).

accumulator compared to the low Cd accumulator. The total Cd uptake was 1.8 times higher in the grafted cultivar than in the low Cd accumulator ($p < 0.05$).

Despite the differences in Cd concentrations among the cultivars, we observed that the internal translocation factors (ITFs) did not differ among them (Table 2). Only the $\text{ITF}_{\text{IF3leaf-IF2leaf}}$ differed significantly between the high Cd accumulator (0.93 ± 0.17) and the grafted (1.63 ± 0.36) cultivar. In conclusion, the differences in Cd concentrations in plant parts among the three cultivars are mainly related to differences in Cd uptake rather than differences in internal distribution.

3.3. Isotope composition of soil and organs of the three cultivars

The isotope compositions of total Cd in the topsoils of the three plots did not differ among cultivars and were, on average, -0.32 ± 0.05 ‰ (mean \pm SD, $n = 8$, Fig. 3). A shift towards heavier isotopes was observed from the total topsoil Cd soil pool to the $\text{Ca}(\text{NO}_3)_2$ -extractable pool ($\delta^{114/110}\text{Cd}_{\text{extractable topsoil}} = -0.17 \pm 0.06$ ‰ [mean \pm SD, $n = 7$]). We estimated the average weighted isotope composition of the entire cacao tree by biomass assumptions for the three cultivars (see section 2.4), and observed that all trees preferentially absorbed the lighter Cd isotopes from the $\text{Ca}(\text{NO}_3)_2$ -extractable Cd soil pool ($\Delta^{114/110}\text{Cd}_{\text{tree-topsoil extract}} = -0.18 \pm 0.04$ ‰, Table 3 (mean \pm SD, $n = 7$)).

The Cd isotope composition followed the order roots \leq nibs, F2 leaves \leq branches \leq pod husks $<$ IF2 and IF3 leaves for the high and grafted cultivars, and nibs \leq roots \leq branches \leq pod husks $<$ IF2 and IF3 leaves for the low cultivar (Fig. 3). In more detail, the enrichment in heavy isotopes from root to branches was significant for the high Cd accumulator ($\Delta^{114/110}\text{Cd}_{\text{branch-root}} = 0.26 \pm 0.02$ ‰, Table 3), but not for the low Cd accumulator ($\Delta^{114/110}\text{Cd}_{\text{branch-root}} = 0.08 \pm 0.05$ ‰), and the grafted cultivar ($\Delta^{114/110}\text{Cd}_{\text{branch-root}} = 0.10 \pm 0.04$ ‰). The mature leaves (IF2 and IF3) were enriched in heavy isotopes compared to the branches in all cultivars. Yet, the high Cd accumulator showed a smaller enrichment of heavy isotopes from branch to IF3 leaves than the low Cd accumulator and the grafted cultivar (Table 3).

In the fruit, the general trend was that the isotope composition of the nib was lighter than that of the pod husk. The pod husk was isotopically similar to the branch (low Cd accumulator) or slightly heavier (high Cd accumulator) whereas the nib was similar (high Cd accumulator) or lighter (low Cd accumulator) than the branch. Finally, the nibs were

isotopically lighter than the IF2 and IF3 leaves, with larger $\Delta^{114/110}\text{Cd}_{\text{nib-IF3}}$ leaves in the low Cd accumulator (-0.54 ± 0.07 ‰) than in the high Cd accumulator (-0.24 ± 0.06 ‰). The young developing leaves (F2) had a similar isotope composition to the nibs in the low Cd accumulator and in the grafted cultivar and could not be sampled in the high Cd accumulator.

3.4. The uptake and distribution of nutrients relative to Cd

Using the Cd and nutrient (P, Cu, S, K, Zn, Mn, Ca, Mg) concentrations measured in each organ (Table 1 and Table S2) and the mass balance estimations, we calculated the total uptake of Cd and nutrients and evaluated the differences between cultivars statistically (Fig. 2 and Table S3). The total tree uptake of Cd and Mn was significantly higher in the high Cd accumulator compared to the low Cd accumulator. The mature leaves were the organs with the highest Mn concentration in all cultivars, ranging from 239 to 990 g kg^{-1} in the low Cd accumulator and grafted cultivar and from 1776 to 1863 g kg^{-1} in the high Cd accumulator (Table S2). The Mn internal translocation factor between IF2/IF3 leaves and roots, ranged between 12 and 32. For K, and S, the trend was inverse: total tree K was higher in the grafted cultivar than in the high Cd accumulator, and total S was higher in the low Cd accumulator than in the high Cd accumulator. For all other nutrients measured, the total uptake was similar among cultivars.

Nutrient/Cd ratios provided information on the separation between nutrients and Cd when they are translocated from one organ to another. To compare the nutrient/Cd ratios among cultivars and among organs, we performed a log transformation of this ratio to comply with the ANOVA assumptions (Table S4). The ANOVA tests revealed that for all nutrients, except S and Mn, the log nutrient/Cd concentration ratio was similar between roots and branches across cultivars (Table S4). Thus, for P, Ca, K, Zn, and Mg there was no significant separation between Cd and these nutrients during their transport from roots to branches. In contrast, there was a large separation of Mn from Cd, with relatively more Mn than Cd being transported than Cd from root to the branches, in all cultivars. The opposite was observed for S, with less S than Cd being exported relative to Cd from roots to branches, in the high Cd accumulator and in the grafted cultivar.

The IF2 and IF3 leaves of the high and low Cd accumulator, and the IF3 leaves of the grafted cultivar showed a marked enrichment of Mn relative to Cd compared to other organs (especially the branch and roots) (Table S4). Thus, there was a significant separation of Mn from Cd in their transport from roots to the leaves. Additionally, in the high Cd accumulator, the IF2 and IF3 leaves also showed an enrichment in Zn and Mg relative to Cd compared to branch and roots (Table S4).

3.5. Elemental micro-localization in the leaves and branches in the high and low Cd accumulator

We used LA-ICP-MS to image the elemental distribution around secondary veins of IF3 leaves from both the low and high Cd accumulator (Fig. 4). In the optical images, we could distinguish some leaf structures, such as the vascular bundle (containing the xylem and phloem), surrounded by parenchymatous cells (Viet Ha et al., 2016). In the low Cd accumulator, we additionally imaged a gland, which may be involved in storage of mucilage (Garcia et al., 2014), and part of the leaf blade, with the mesophyll and epidermis.

In the low and high Cd accumulator, the Cd enrichment in the parenchyma of the secondary vein compared to the vascular bundles was similar. In both cultivars, the strongest colocalization was between Cd and Mn. In the images of the low Cd accumulator, Cd also colocalized with P and Zn, but not in the images of the high Cd accumulator. Finally, we observed in the low Cd accumulator, an accumulation of Cd around a gland.

We previously characterized branch samples of the high Cd accumulator by LA-ICP-MS (Blommaert et al., 2022). Similar images were

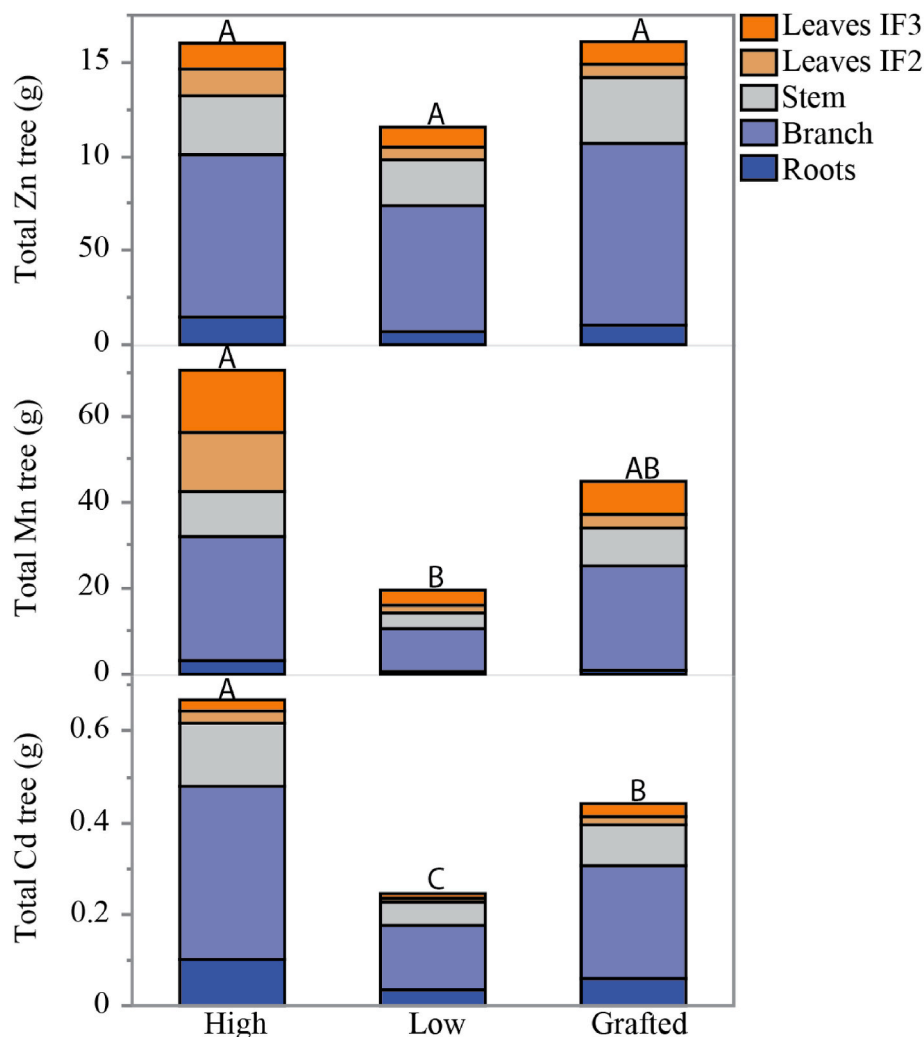


Fig. 2. Total Cd, Mn, and Zn (g) in the organs of the trees calculated with assumptions on tree biomass (see 2.4). The Cd, Mn, and Zn concentrations in the stem were estimated to be equal to the concentrations in the branches. Letters A, B, and C denote different statistical groups among cultivars for total Cd uptake (Table S3). IF2 (inter flush-2) leaves are mature green leaves with green stem, IF3 leaves are mature green leaves with brown stem. The biomass of the fruits and developing (F2) leaves was negligible compared to the total tree biomass. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

obtained for the low Cd accumulator (T3 and T15, Figs. S1 and S2, pixel size: 10 μm). Like in the high Cd accumulator, Cd was clearly enriched in the bark versus the wood. More in detail, Cd was enriched in the phloem rays and the periderm compared to other regions. In the regions indicated in Fig. S1, Cd colocalized most strongly with Mn followed by Zn and Ca, and less with P, Cu and Ni, except for the periderm (Table S5). In the latter, there was a high co-localization with P as well. The maps showed a clear accumulation of Mn and Cu in the periderm which was less pronounced for Cd. Just like for Cd, there was an enrichment of Mn, Zn and Ca around the big secretory vessels in the pith and bark, which have been reported to transport mucilage in cacao trees (Angyalossy et al., 2016; Brooks and Guard, 1952) (Figs. S1 and S2). Thus, to summarize, images of the branches of the high and low Cd accumulator showed a similar distribution of Cd at the microscale.

4. Discussion

4.1. Soil characteristics that determine Cd bioavailability are similar in the three soil plots

Total soil Cd, pH and SOC were similar in all plots. A meta-analysis of $n = 12$ studies in Latin America and the Caribbean indicated that these

parameters explained >41% of the variance in bean Cd concentrations (Vanderschueren et al., 2021). As these parameters and the Ca $(\text{NO}_3)_2$ -extractable Cd did not differ significantly in the topsoil and subsoil of the three plots (Table S1), we assume that the Cd soil bioavailability was similar in the plots of all cultivars. Likewise, Mn bioavailability was similar in all plots, as indicated by ammonium oxalate extractions and Ca $(\text{NO}_3)_2$ -extractions. One exception was the Ca $(\text{NO}_3)_2$ -extractable Mn that was lower for the grafted cultivar than for the high Cd accumulating cultivar. It should be noted that the topsoil of the grafted cultivar showed higher CEC and ammonium extractable Fe and Al than the other cultivars. An increase in sorbing phases may decrease Cd and Mn bioavailability (Smolders and Mertens, 2013). In our study, higher ammonium extractable Fe and Al did not correspond to a significant decrease in Ca $(\text{NO}_3)_2$ -extractable Cd, but may have contributed to a slight reduction in Ca $(\text{NO}_3)_2$ -extractable Mn in the plot of the grafted cultivar compared to the other plots.

4.2. Cadmium uptake differs among cultivars

The high Cd accumulator exhibited a particularly high Cd transfer from soil to the leaves ($\text{TF}_{\text{leaf IF2-soil}} = 13.1$) (Blommaert et al., 2022). The transfer factors for the low ($\text{TF}_{\text{leaf IF2-soil}} = 5.2$) and grafted (TF_{leaf}

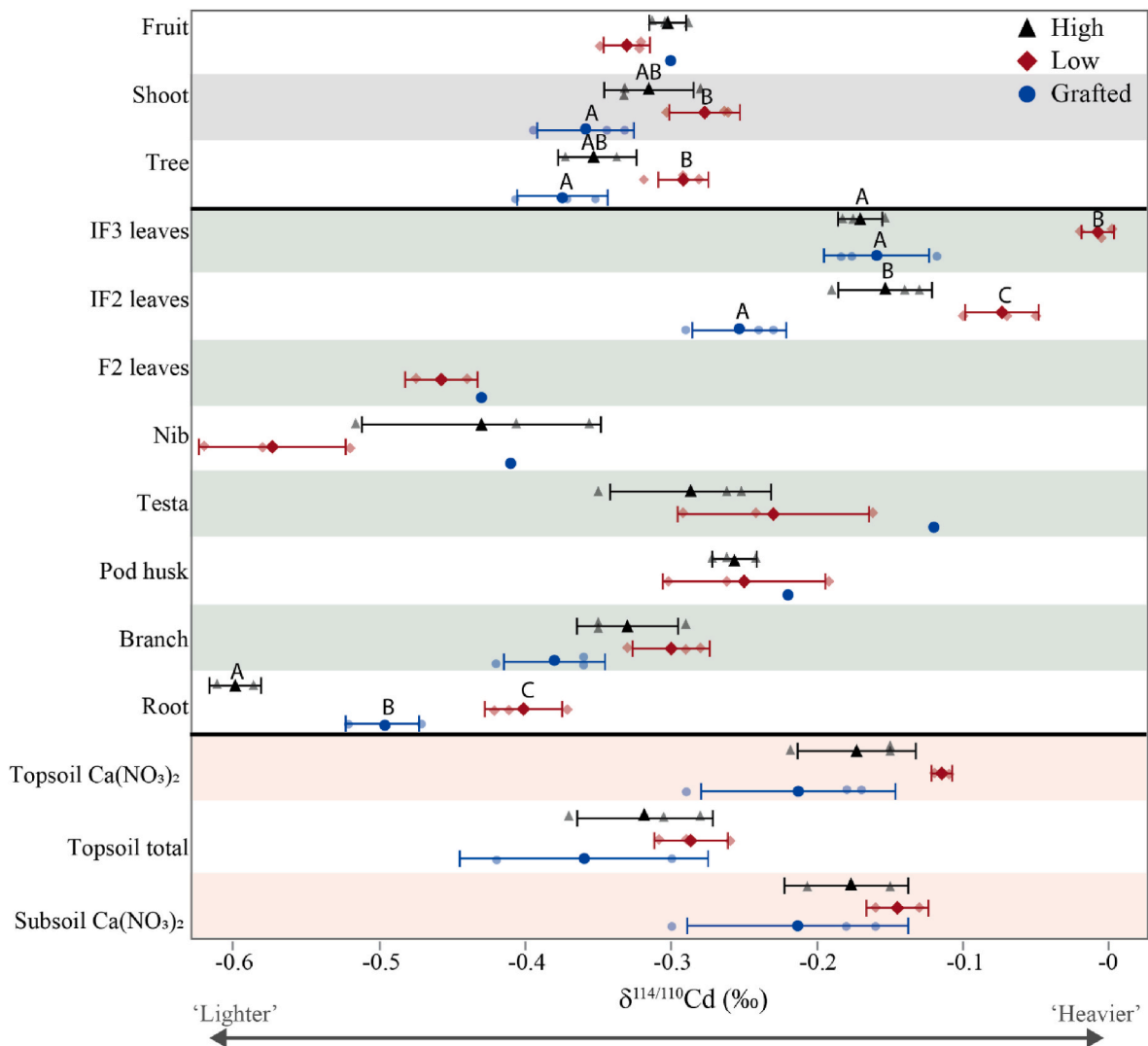


Fig. 3. Cd isotope compositions ($\delta^{114/110}\text{Cd}$) for the soil and different cacao plant organs. The measurements of each biological replicate are depicted with transparent markers and the mean of the biological replicate with a non-transparent marker. The error bars represent the standard deviation (SD), calculated with the biological replicates, if available. Letters A, B, C denote different statistical groups in Cd isotope composition of individual tree parts/organs among cultivars with the post hoc Tukey HSD test ($p < 0.05$). Topsoil total: total digestion (aqua regia with subsequent HF digestion) and 0.05M $\text{Ca}(\text{NO}_3)_2$ -extract (5g soil: 50 mL $\text{Ca}(\text{NO}_3)_2$). The isotope composition of the tree, shoot and fruit was calculated based on biomass assumptions (see 2.4). Samples on the left side of the graph are depleted in ^{114}Cd (isotopically 'lighter') relative to samples on the right side of the graph (isotopically 'heavier').

$\text{IF}_{2\text{-soil}} = 7.2$) cultivar were closer to the averages $\text{TF}_{\text{leaf-soil}}$ (4.5, 3.4, 4.9, 7.1) observed in four surveys in Ecuador and Honduras (Vanderschueren et al., 2021). Thus, the chosen cultivars represented the variability in leaf Cd concentrations observed among cacao trees.

Despite similar soil properties, there was a clear difference in Cd concentrations among the three different cultivars. The high Cd accumulator and the grafted took up 2.7 and 1.8 times more total Cd than the low Cd accumulator (Fig. 2, Table S3). From all nutrients investigated, Mn showed the most similar pattern. The high Cd and grafted cultivar accumulated in average 3.6 and 2.3 more Mn than the low Cd cultivar, respectively. Zinc showed the same trend, but less pronounced and statistically not significant (Fig. 2, Table S3). Note that differences in Cd uptake amongst cultivars cannot be explained by differences in bioavailable Cd content, as has been discussed in section 4.1, thus the differences in Cd uptake must result from genotype differences.

To trace the processes that caused these differences in Cd uptake among the three cultivars, we analyzed the differences in Cd isotope compositions of the trees compared to the soil bioavailable Cd. All trees in our study preferentially absorbed light Cd isotopes from the soil $\text{Ca}(\text{NO}_3)_2$ -extractable fraction ($\Delta^{114/110}\text{Cd}_{\text{tree-topsoil extract}} = -0.18 \pm 0.04$

‰, $n = 3$ cultivars). This is consistent with previous observations in other plant species (Wiggenhauser et al., 2022). Notably, a study on cacao seedlings reported a similar extent of Cd isotope fractionation from the hydroponic solution to the plant ($\Delta^{114/110}\text{Cd}_{\text{seedling-hydroponic solution}} = -0.22 \pm 0.08$ ‰, $n = 19$ cultivars) (Moore et al., 2020). This study also showed that the TcNRAMP5-transporter from the Natural Resistance-Associated Macrophage Proteins (NRAMP5) family preferentially transports light Cd isotopes (Moore et al., 2020). The gene *TcNRAMP5* is constitutively expressed in cacao roots, and is an ortholog of *OsNRAMP5*, responsible for Cd and Mn uptake in rice (Ullah et al., 2018). Hence, the combination of concentration and isotope data in this study supports the hypothesis that Cd uptake can be related to Mn uptake through the TcNRAMP5 transporter. Gene expression studies would be necessary to further test the role of TcNRAMP5 on Cd uptake in low and high Cd accumulating cultivars, ideally combined with analyses of the regulation and structure of TcNRAMP5 transporters.

Table 3

Differences in isotope composition ($\Delta^{114/110}\text{Cd}_{\text{A-B}} (\%) = \delta^{114/110}\text{Cd}_{\text{A}} - \delta^{114/110}\text{Cd}_{\text{B}}$) between plant organs or between the tree and the soil for the three sampled cultivars.

	$\Delta^{114/110}\text{Cd}_{\text{A-B}}(\%)$				Group	Low				Group	Grafted				Group
	n	Mean	SD	95% CI		n	Mean	SD	95% CI		n	Mean	SD	95% CI	
branch-root	2	<i>0.26*</i>	<i>0.02</i>	0.03/0.48	a	3	0.08	0.05	-0.05/0.21	b	3	0.10	0.04	0.00/0.19	b
pod husk-branch	3	<i>0.07*</i>	<i>0.02</i>	0.02/0.13		3	0.04	0.05	-0.08/0.17		1	0.14			
nib-branch	3	<i>-0.10</i>	<i>0.06</i>	-0.25/0.05		3	-0.26*	0.06	-0.40/-0.11		1	-0.05			
IF2 leaf-branch	3	<i>0.18*</i>	<i>0.04</i>	0.08/0.27		3	0.23*	0.05	0.10/0.35		3	0.13	0.06	-0.01/0.26	
IF3 leaf-branch	3	<i>0.15*</i>	<i>0.02</i>	0.11/0.18	b	3	0.29*	0.02	0.25/0.32	a	3	0.22*	0.05	0.11/0.34	a
pod husk-IF2 leaves	3	<i>-0.10*</i>	<i>0.03</i>	-0.17/-0.04	a	3	-0.18*	0.01	-0.20/-0.17	b	1	0.01			
pod husk-IF3 leaves	3	<i>-0.07*</i>	<i>0.01</i>	-0.10/-0.04	a	3	-0.24*	0.04	-0.33/-0.15	b	1	-0.12			
nib-IF2 leaves	3	<i>-0.28*</i>	<i>0.10</i>	0.03/0.52		3	-0.48*	0.10	-0.23/-0.73		1	-0.18			
nib-IF3 leaves	3	<i>-0.24*</i>	<i>0.06</i>	0.09/0.41		3	-0.54*	0.07	0.36/0.72	a	1	-0.31			
nib-F2 leaves						2	-0.09	0.08	-0.64/0.81						
testa-nib	3	<i>0.14*</i>	<i>0.03</i>	0.07/0.22		3	0.33	0.14	-0.02/0.67		1	0.29			
pod husk-nib	3	<i>0.17</i>	<i>0.08</i>	-0.01/0.36		3	0.30*	0.06	0.05/0.55		1	0.19			
tree-topsoil [§]	2	<i>-0.05</i>	<i>0.04</i>	-0.45/0.34		3	-0.01	0.02	-0.04/0.03		2	-0.03	0.11	-1.04/0.98	
tree-topsoil extract [§]	2	<i>-0.20</i>	<i>0.03</i>	-0.44/0.40		3	-0.21*	0.06	-0.03/-0.07		3	-0.16	0.08	-0.35/0.02	
shoot-root [§]	2	<i>0.27*</i>	<i>0.02</i>	0.08/0.46	a	3	0.10	0.05	-0.02/0.23	b	3	0.12*	0.04	0.03/0.21	b
fruit-branch [§]	3	<i>0.03</i>	<i>0.02</i>	-0.03/0.08	a	3	-0.03	0.01	0.00/0.06	b	1	0.06			a

The data in italic is published in Blommaert et al. (2022). Mean and SD were calculated with biological replicates: $\Delta^{114/110}\text{Cd}_{\text{A-B}} (\%)$ was calculated for each biological replicate (individual tree), and subsequently averaged per cultivar (paired data). Letters a, b denote different statistical groups with the posthoc Tukey HSD test ($p < 0.05$) among cultivars. *The isotope composition between two plant parts is significantly different when the 95% confidence interval (CI) does not encompass 0 which is indicated with *.

[§]The isotope composition of the tree, shoot and fruit was calculated based on biomass assumptions (see 2.4).

The topsoil was digested with aqua regia and HF; the topsoil extract was a 0.05 M $\text{Ca}(\text{NO}_3)_2$ -extract (5 g soil: 50 mL $\text{Ca}(\text{NO}_3)_2$).

4.3. Cd, Cd isotope distribution, and Mn/Cd separation: similarities among cultivars

For all cultivars, the root-to-shoot Cd translocation was within a similar range, with $\text{ITF}_{\text{branch-root}}$ values ranging from 1.9 to 2.2 and $\text{ITF}_{\text{leaf-root}}$ values from 0.91 to 1.33 (Table 2). ITFs and Cd mass distributions indicated a similar distribution in root and shoot organs across all cultivars (Table 2, Fig. 2). Hence, there were no cultivar-related differences in Cd distribution in our study. Field studies have observed differences in Cd distribution among cultivars, yet these studies investigated more cultivars and were potentially influenced by soil Cd heterogeneities (Arévalo-Gardini et al., 2017; Lewis et al., 2018; Engbersen et al., 2019).

In addition to this similar distribution at the plant scale, the high and low cultivars showed a similar microscale Cd distribution in leaves (Fig. 4) and branches (Figs. S1 and S2, and (Blommaert et al., 2022)). Among nutrients, Mn showed the highest colocalization with Cd at the microscale (Table S5). In both the high and the low Cd cultivars, the images suggested a xylem to phloem transfer of Cd through the rays of branches (as described in Van Bel, 1990) and a Cd storage in phloem and periderm of branches. We observed as well clear enrichments in Cd around secretory gland in cacao leaf image of the low Cd accumulator and secretory vessels in branches of both cultivars. It is known that these structures produce and store mucilage in cacao tree (Brooks and Guard, 1952; Angyalossy et al., 2016). Thus, the microscale images indicate that Cd is present in secretory cells around mucilage cavities in branches and leaves, although the Cd concentrations in cacao mucilage inside the fruits have been reported to be negligible (Vanderschueren et al., 2020).

The Cd isotope composition followed the order roots \leq nibs, F2 leaves \leq branches \leq pod husks $<$ IF2 and IF3 leaves for the high and grafted cultivars, and nibs \leq roots \leq branches \leq pod husks $<$ IF2 and IF3 leaves for the low cultivar. Thus, in the three cultivars, Cd translocation along the xylem pathway (roots-branches-leaves) was associated with an enrichment in heavy Cd isotopes (Fig. 3). Interestingly, the Mn/Cd ratio in the root, branches, IF2, IF3 leaves increased in the same order as the Cd isotope composition (Table S4, Fig. 5). Thus, Cd was progressively retained along the xylem pathway compared to Mn, leading to a Mn/Cd separation in low and high Cd cultivars. The xylem to phloem transfer of Cd in the branches discussed above likely participates to the retention of

light Cd isotopes.

All cultivars showed an enrichment in heavy isotopes between the mature IF2 and IF3 leaves (xylem sinks) and the branches. The low Cd accumulator showed a significant enrichment in light isotopes in nib and developing F2 leaves (phloem sink), relatives to branches. Consequently, the nibs and F2 leaves of the low Cd accumulator largely differ from the linear trend between the Cd isotope compositions and the log (Mn/Cd) (Fig. 5). These data suggest an enrichment in light isotopes of the phloem compared to the xylem and a preferential transfer of light isotopes during the xylem to phloem transfer in branches. These results corroborate the previous interpretations of the Cd isotope compositions in the high cultivar (Blommaert et al., 2022). Finally, the isotope compositions of testa and pod husk were close to that of the branches (Fig. 3). This observation suggests that, for all cultivars, the maternal tissues (testa and pod husk) receive Cd from both the xylem and phloem.

4.4. Differences in Cd isotope composition between cultivars

Although, the partitioning of Cd in the tree (as given by the internal translocation factors) was similar among cultivars, the differences in isotope compositions among cultivars suggested small differences in Cd translocation processes. For instance, in the high Cd accumulator, the preferential retention in light isotopes in roots compared to shoots was more pronounced than in the low Cd accumulator and the grafted cultivar (Table 3). The significant enrichment in light Cd isotopes between roots and shoots observed in the high Cd accumulator was previously found in cereals (Wiggenhauser et al., 2021), and various cultivars of cacao (Moore et al., 2020). It can be due to membrane transport and binding of Cd to thiol-containing organic ligands (Aucour et al., 2023). In cacao roots of the high Cd accumulator, both Cd binding with S-ligands and binding with Ca-oxalate crystals have been observed (Blommaert et al., 2024a). Further study of the Cd isotope fractionation associated with Cd binding to oxalate is necessary to understand the effect of ligand-type on isotope fractionation inside the roots. Theoretical calculations predict a slight enrichment in heavy isotopes of Cd bound to citrate, compared to Cd-aqua-complexes (Zhao et al., 2021). Hence, a similar trend may be expected for oxalate. Following this logic, the isotope pattern in the roots indicates that Cd was probably more bound with O-ligands in the roots of the low and grafted cultivar, while

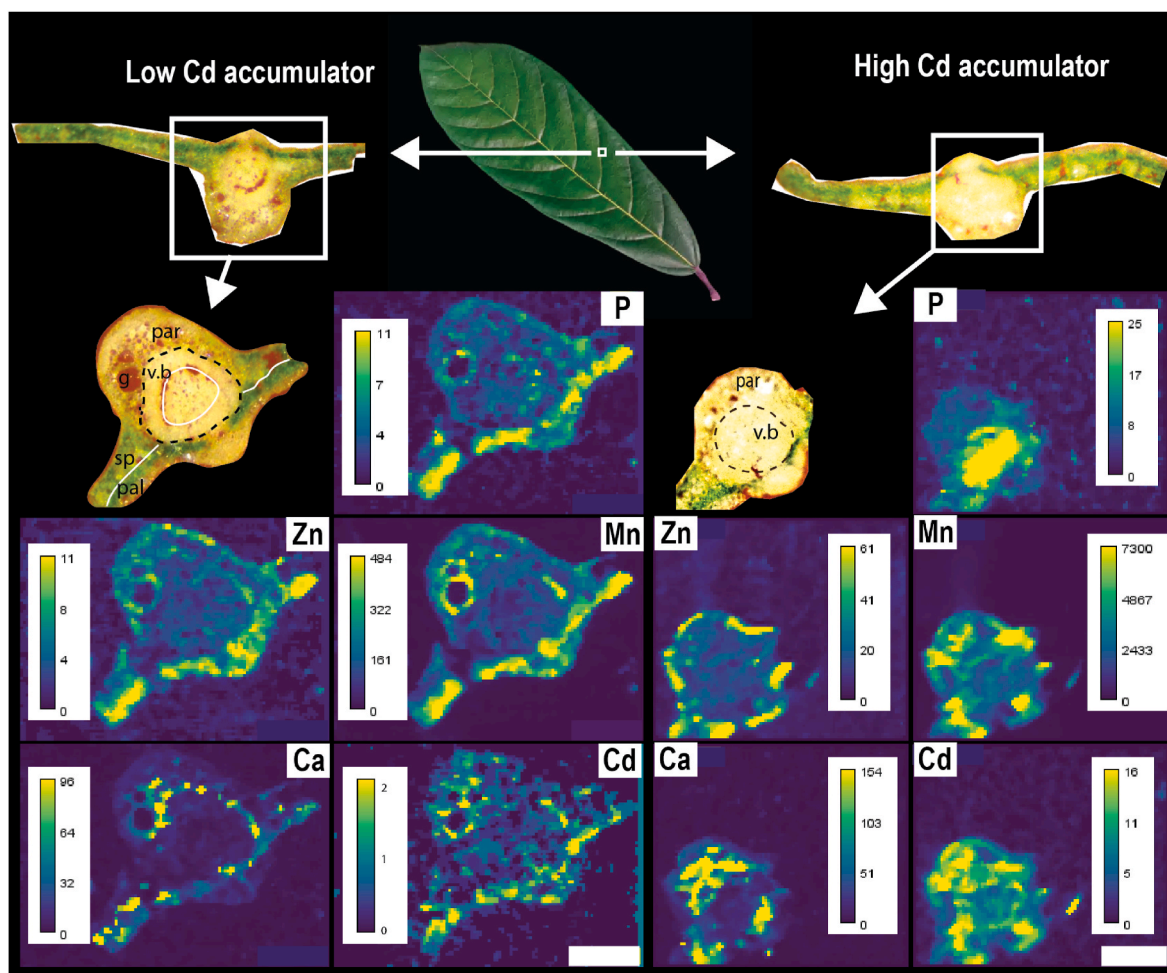


Fig. 4. Microscope images and LA-ICP-MS elemental maps of a freeze-dried cross section of secondary veins of IF3 leaves of the high and low Cd accumulator. The intensity is expressed in total counts (cts). The color scale is adjusted to the range of each element. V.b.: vascular bundle, par: parenchymous tissue, pal: palisade mesophyll, sp: spongy mesophyll, g: gland. Scalebar, given in the Cd map in white, is 200 μm . (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

in the roots of the high Cd accumulator more S-ligands were prevalent.

In the high Cd accumulator, the enrichment in heavy isotopes between the IF3 leaves (xylem sink) and the branches was smaller compared to the low and grafted cultivar. The depletion in heavy isotopes between the nibs (phloem sink) and the branches was also less pronounced in the high Cd accumulator (Fig. 3). Here, the smaller selectivity for light isotopes along the transpiration stream goes with a slightly larger separation of Cd from Mn as indicated by the higher log (Mn/Cd) of IF2 leaves (Table S4), and corresponds with a relatively lower log(Mn/Cd) in the nibs. These differences suggest that the mechanisms of Cd translocation from branches to leaves and nibs slightly differ between cultivars. The preferential retention of Cd in the branch with binding to O-ligands (such as Ca-oxalate (Blommaert et al., 2024), can favor a retention of heavy Cd isotopes (as discussed above). Another process that could fractionate Cd isotopes, is the transfer through the secretory cavities in the bark and pith, as observed in the images (Fig. 4, S1, and S2). To better interpret the isotope results, sampling and analysis of the mucilage would be valuable, as well as further speciation studies in the different organs of the low and grafted cultivar.

4.5. Cultivar-related differences in Cd uptake and translocation: the potential for mitigating Cd in cacao

Our study revealed notable differences in Cd accumulation between

a high Cd accumulator (NA 312 of the genetic group *Nanay*) and a low Cd accumulator (CL 10/3 of the genetic group *Refractario*). The primary difference in Cd concentrations in the nibs of these cultivars was attributed to variations in Cd uptake rather than differences in distribution and micro-distribution in branches and leaves. Notably, Cd and Mn were highly correlated in uptake. This correlation suggests that membrane proteins responsible for Mn uptake may play a role in Cd uptake. For genetic breeding studies, we thus recommend to target genes for Mn uptake. Further along the translocation pathway, we observed differences in the mechanisms of root-to-shoot translocation, reflected in the differences in apparent isotope fractionation from branches to leaves and nibs. It should however be noted that these differences were not affecting internal and micro-distribution of Cd in the trees. For all cultivars, there was a significant separation of Mn from Cd in their transport from roots to the leaves. Hence, for genetic approaches, genes that encode for Cd retention and Mn translocation can be further explored.

The grafted cultivar served as a valuable comparison to the high and low Cd accumulating cultivars. Moreover, these trees revealed an effect of the scion on Cd uptake, as the low Cd accumulator accumulated less Cd than the grafted, despite both having the same scion. There was again a strong correlation between Cd and Mn uptake. The combination of a rootstock of a low Cd accumulating cultivar with an agronomically desirable scion could effectively mitigate Cd in cacao. Yet, this study falls short to prove directly rootstock effects as it only compares one grafted cultivar with a non-grafted cultivar. Nevertheless, this study

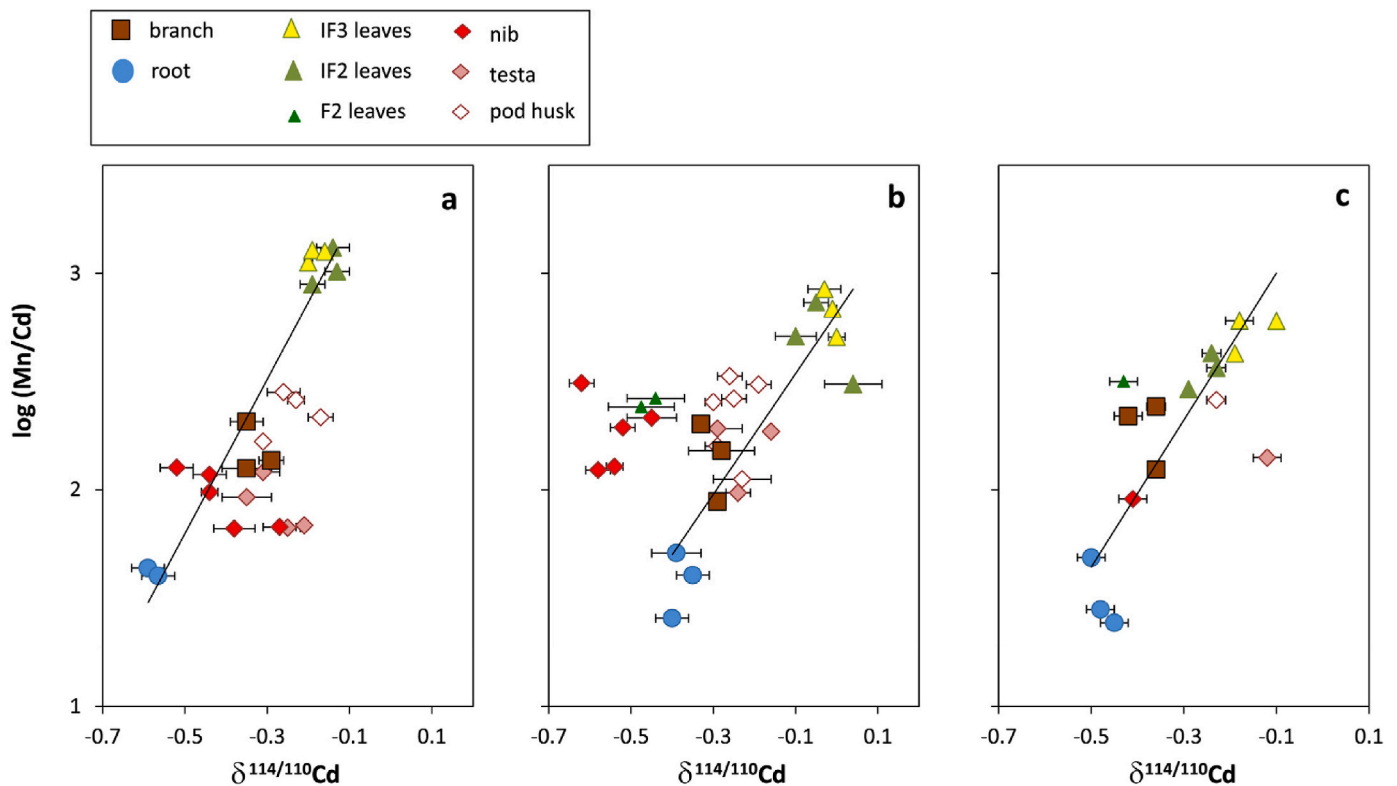


Fig. 5. Log Mn/Cd ratio as a function of the Cd isotope composition for the cacao organs. An increased separation of Mn versus Cd along the transpiration pathway (roots - branches - leaves IF2 and IF3) is related to a heavier isotope composition in the high Cd (a), low Cd (b) and grafted (c) cultivars. In more detail, there is a larger separation of Mn from branch to leaves in the high Cd cultivar together with a smaller enrichment in heavy isotopes. The fruit organs (pod husk, testa, nib) were excluded from the regression fit. The equation of the regression fit between log (Mn/Cd) and Cd isotope composition is for a): $y = 3.56x + 3.58$, $r^2 = 0.91$, $n = 10$; for b): $y = 3.40x + 3.34$, $r^2 = 0.79$, $n = 11$ and for c): $y = 2.79x + 2.81$, $r^2 = 0.80$, $n = 11$. The error bar for the isotope composition is given by replicate measurements of each biological sample (generally at least three). For log (Mn/Cd), the error bar was calculated using error propagation and analytical uncertainty on Mn and Cd concentrations; it falls within the symbols used.

indicates that there is potential in rootstock effects and that existing data of Mn uptake across different grafts can be explored for Cd mitigation.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.plaphy.2025.109511>.

Data availability

Data will be made available on request.

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